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The TVA retroviral gene transfer system allows for the examination of multiple genetic lesions in vivo without the need to create and breed individual transgenic lines. The system is based on the use of the RCAS virus (an avian leukosis virus vector of subgroup A) to deliver genes to mammalian cells or tissues that have been engineered to produce the avian viral receptor TVA. Transgenic mice have been generated to express *TVA* in the mammary gland. Mammary tumors can be induced in these mice by in vivo infection of mammary glands with virus encoding polyoma middle T antigen. Mammary cells, isolated from the TVA mice that have been bred to *p53* nullizygosity, can be infected ex vivo with RCAS vectors expressing oncogenes, and the transplantation of the infected cells into the fat pad of non-transgenic mice results in rapid development of mammary tumors. This somatic gene delivery system may be useful for dissecting genetic interactions that operate in breast cancer.

In addition, the investigator has succeeded in two additional research projects, the development of a preclinical model for treating breast cancer with CCI-779 (a rapamycin analog) and a study showing strong evidence for stem cell neoplasia in MMTV-*Wnt-1* transgenic mice.

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Mammary-Specific Gene Transfer For Modeling Breast Cancer

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INTRODUCTION

Breast cancer is a complex disease involving many genetic lesions including mutations in *p53*, *BRCA1,* and *BRCA2*, *PTEN/MMAC1*, *neu/ErbB2/HER2*, *ErbB1/EGFR*, *PRAD-1/cyclin D1*, *Mdm2*, and *c-myc*. In general, several genetic factors are necessary to drive a normal breast cell to neoplasia, but it is poorly understood how they collaborate to transform breast cell. Conventional transgenic and knockout approaches are inflexible in examining genetic interactions. My research is designed to use a viral gene transfer system (Federspiel et al. 1994) to overcome some of the limitations of these conventional techniques. This system is based on the ability of avian leukosis virus subgroup A (ALV-A) to transduce TVA+ mammalian cells without the production of infectious viral particles (reviewed by Fisher et al. 1999). Consequently, after generating a single transgenic line expressing *tv-a* in a target tissue, individual and combinatorial effects of any oncogenic genes can be examined using ALV-A as a delivery vehicle. This system has been demonstrated to work in modeling gliomas (Holland et al. 1998; Holland and Varmus 1998; Holland et al. 2000) and ovarian cancer (Orsulic et al. 2002). In the following text, I will summarize my efforts to adapt this system to model the genetic interactions in breast cancer.

BODY

Task 1. Establish mammary-specific gene transfer methods (months 1-6).

This section has been finished and reported in previous annual reports.

Task 2. Introduce protooncogenes/oncogenes and dominant-negative TSGs into mammary glands in an effort to produce tumors (months 3-24).

A number of RCAS viruses have been made to express genes implicated in human breast cancer, including a mutant form of *HER2/neu*, *c-myc*, *Akt*, and *cyclin D*1. I have succeeded in two approaches to induce mammary tumors using the TVA technology.

1). Inducing mammary tumors using transplantation of primary mammary cells transduced with oncogenes.
p53 is mutated in about 40% of human breast cancer cases; therefore, it may be easier to induce mammary tumors using this viral method if p53 is already mutated in the target cells. p53 deficient mice die of tumors other than mammary origin in a few months after birth, so the window of time left for induction of mammary tumors in these mice is quite small. However, if p53 null mammary cells are manipulated genetically ex vivo and transplanted into p53 wild type mice, the outcome of genetic alterations can be read without the pleiotropic effects of the loss of p53 on the host. In addition, viral infection reaches a higher efficiency and can be better monitored in culture. For these reasons, I chose transplantation of MA/p53-/- mammary cells after infection in

culture with oncogenic viruses. Infection of these cells with RCAS expressing an activated mutant of K-Ras (K-RasG12D) led to appearance of mammary tumors in two months following orthotopic transplantation. Other oncogenic viruses are being tested using this approach.

2). Inducing mammary tumors by a direct injection of oncogenic viruses into surgically exposed mammary glands of TVA transgenic mice. As a proof of principle, I have demonstrated that in vivo infection of mammary glands of TVA transgenics with the gene encoding polyoma middle T antigen (PyMT) led to development of mammary tumors that are similar to those seen in transgenic mice expressing PyMT from the LTR of MMTV (Guy et al. 1992). As anticipated, PyMT protein can be detected in these tumors using a Western assay. Since it takes nearly one year for the infected mice to develop tumors, additional genetic events must be required. Experiments are underway to induce a more rapid tumor development using RCAS-PyMT and other oncogenic viruses in mice that are conditionally mutated for a tumor suppressor gene such as p53 (see Task 5 for more detail).

Task 3. Generate a mouse line carrying a floxed *Brca2* allele in collaboration with Anthony Wynshaw-Boris (months 1-12).

This task has been discontinued. The reason have been provided in an earlier report.

Task 4. Delete floxed TSG in mammary glands at targeted times in hopes of generating tumors (months 12-24).

Since it is known now that it takes a long time for mice losing a single tumor suppressor gene to develop mammary tumors (Xu et al. 1999; Kuperwasser et al. 2000), I have decided to combine the effort to induce mammary tumors with that in Task 5.

Task 5. Express protooncogenes/oncogenes and inactivate floxed TSGs in the mammary glands in order to generate tumors (months 24-36).

In order to generate mammary tumors rapidly and to study collaborative effects between tumor suppressor genes and oncogenes, both classes of genetic lesions will be introduced to the same mammary cells using the TVA gene transfer method. I have imported mice carrying floxed *Brca-1* (Xu et al. 1999) or floxed p53 (Jonkers et al. 2001) and have bred the floxed alleles into the MA line. The resulting *tv-a* TG/*Brca*$^{fl/fl}$ or *tv-a* TG/*p53*$^{fl/fl}$ will be used for infections with RCAS expressing an oncogene linked with Cre by IRES so that cells infected will gain an oncogene and lose the function of a tumor suppressor gene. Viral vectors expressing Ras-IRES-Cre or neu-IRES-Cre are being made for infections.

Task 6. Characterize tumors generated in the course of this study (months 24-36).

This section represents future work.

Other achievements not proposed in the approved proposal

1. A preclinical model for use of CCI-779 to treat breast cancer

CCI-779 is a rapamycin analog developed by Wyeth-Ayerst for better intravenous delivery. It inhibits

mTOR (mammalian target of rapamycin), leading to the inhibition of the translation of mitogen-activated proteins, such as c-Myc and Cyclin D1. In preclinical models, CCI-779 has been shown to inhibit the growth of tumors in the uterus and prostate without adverse effects on normal cells such as colon epithelial cells. In addition, it has been shown to inhibit the growth of some human breast cancer cell lines.

To test if CCI-779 has any effect on inhibiting the growth of breast tumors, we treated the tumor-bearing MMTV-*Wnt-1* transgenic mice with daily IV injections of CCI-779. After two days on the drug, mammary tumors in these mice showed reduced proliferation and decreased levels of phosphorylated S6K, a target of mTOR. More importantly, the majority of the tumors stopped growing over the course of two months of treatment, whereas diluent-injected mice had to be sacrificed because the tumors grew too large. In addition, mammary hyperplasia was also significantly reduced, whereas it was widespread in diluent-injected mice.

The finding that some tumors did not respond to CCI-779 suggests that there are additional genetic events that are responsible for resistance or susceptibility to this drug. Micro-array and other experiments are underway to elucidate the factors that may contribute to the resistance/susceptibility of these tumors.

2. Evidence for mammary hyperplasia and neoplasia arising in progenitor cells in the mouse mammary gland of MMTV-*Wnt-1* transgenic mice (manuscript in preparation)

Human breast cancer is generally thought to arise in the terminal end buds of the ductal tree. Several cell types are present at this site including stem cells, immature ductal cells, terminally differentiated ductal cells, and myoepithelial cells. It is unclear if different target cells may contribute to the heterogeneity seen in breast cancer, and it is also unknown which cell types may be the most vulnerable to oncogenesis. Transgenic expression of *Wnt-1* in the mouse mammary gland leads to expansion of epithelial cells expressing markers of progenitor cells. In the resulting tumors, markers of stem cells are expressed, and there exist at least two populations of tumor cells—one expressing the epithelial marker keratin 8 and another expressing the myoepithelial marker, alpha-smooth muscle actin—implying differentiation from a progenitor cell. A significant proportion of myoepithelial cells (albeit normal in cytology) is also detected in mammary tumors from transgenic mice expressing β-catenin and c-Myc, both of which are regulated by Wnt-1; however, very few myoepithelial cells are present in mammary tumors from transgenic mice expressing *neu*, *H-Ras* or polyoma middle T antigen. Therefore, the differentiation state of breast cancers may be dictated by the initiating oncogenetic events. Collectively, these results suggest that mammary progenitor cells may be target cells for oncogenesis and that tumors arising in progenitor cells may be identified by the expression of progenitor cell markers and the presence in the tumor of multiple differentiated cell types derived from the same lineage.

KEY RESEARCH ACCOMPLISHMENTS

A new method to generate mouse models for breast cancer has been developed, based on the use of an avian retroviral vector to transfer genetic mutations to somatic mammary cells.

A new chemotherapeutic drug, CCI-779, potently inhibits mammary tumors in MMTV-Wnt-1 transgenic mice, suggesting that this drug may be useful in treating human breast cancer.

Mammary tumors arising in MMTV-Wnt-1 transgenic mice may originate from mammary stem cells.

REPORTABLE OUTCOMES

1. A new method to generate mouse models for breast cancer is established.
2. Manuscript in preparation: Li et. al., Evidence for mammary hyperplasia and neoplasia arising in progenitor cells in the mouse mammary gland of MMTV-*Wnt-1* transgenic mice.
3. Faculty job: I have accepted an assistant professorship at the Breast Center and Department of Molecular and Cellular Biology, Baylor College of Medicine.
4. Orsulic S, Li Y, Soslow RA, Vitale-Cross LA, Gutkind JS, Varmus HE., Induction of ovarian cancer by defined multiple genetic changes in a mouse model system., Cancer Cell 2002 Feb;1(1):53-62.
5. Poster presentation: Li Y. and Varmus, H. E., Studying genetic interactions leading to breast cancer using a somatic gene transfer system, Gordon Research Conference on Cancer, 8/4-8/9/2002.

CONCLUSIONS

The TVA-mediated gene delivery system developed here for transferring genetic lesions to mouse mammary cells may provide a more flexible method to study genetic interactions in vivo and to rapidly screen for new breast cancer genes.

MMTV-Wnt-1 may be useful as a preclinical model for treating breast cancer with a new class of chemotherapeutic agents targeting the mTOR pathway. The identification of the molecular mechanisms responsible for the resistance and/or susceptibility to CCI-779 in this model may help understand why human breast cancers may be susceptible or resistant to this class of therapeutics.

The compelling evidence for progenitor cell neoplasia in MMTV-Wnt-1 transgenic mice provides strong support for the hypothesis that breast cancer may arise in stem cells. The risk to breast cancer may be reduced if the mammary stem cell population can be suppressed, reduced, or eliminated.

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